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Original Article

# ACE Score Identifies HBeAg-negative Inactive Carriers at a Single-point Evaluation, Regardless of HBV Genotype

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## Abstract

***Background and Aims:*** Hepatitis B virus (HBV) biomarkers have been used for a better categorization of patients, even though the lack of simple algorithms and the impact of genotypes limit their application. Our aim was to assess the usefulness of noninvasive markers for the identification of HBV inactive carriers (ICs) in a single-point evaluation and to design a predictive model for their identification. ***Methods:*** This retrospective-prospective study included 343 consecutive HBeAg-negative individuals. Clinical, analytical, and virological data were collected, and a liver biopsy was performed if needed. Subjects were classified at the end of follow-up as ICs, chronic hepatitis B and gray zone.A predictive model was constructed, and validated by 1000-bootstrap samples. ***Results:*** After 39 months of follow-up, 298 subjects were ICs, 36 were chronic hepatitis B CHB, and nine were gray zone. Eighty-nine (25.9%) individuals required a liver biopsy. Baseline HBV DNA hazard ratio (HR) 6.0, $p<0.001$), HBV core-related antigen (HBcrAg) (HR 6.5, $p<0.001$), and elastography (HR 4.6, $p<0.001$) were independently associated with the IC stage. The ACE score (HBV DNA, HBcrAg, elastography), obtained by bootstrapping, yielded an area under the receiver operating characteristics (AUROC) of 0.925 (95% CI: 0.880–0.970, $p<0.001$) for identification of ICs. The AUROC for genotype D was 0.95, 0.96 for A, 0.90 for E, and 0.88 for H/F. An ACE score of <1 had a positive predictive value of 99.5%, and a score ≤12 points had a diagnostic accuracy of 93.8%. ***Conclusions:*** Low baseline HBV DNA, HBcrAg, and liver stiffness were independently associated with the IC phase. A score including those variables identified ICs at a single-point evaluation, and might be applied to implement less intensive follow-up strategies.

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## Introduction

Chronic hepatitis B infection affects 296 million people worldwide according to the World Health Organization.[1] The course of chronic hepatitis B infection is described in different phases as a result of complex and dynamic interactions between the host immunity and the viral particles.[2] Hepatitis B e antigen (HBeAg)-negative stage represents the vast majority of chronic HBV infections in Western countries.[3,4] The risk of cirrhosis, liver cancer, decompensation, and liver-related death dramatically varies throughout the HBeAg-negative infection phases, which are not always consecutive and whose duration may differ among individuals. Assessment of HBV infection by alanine aminotransferase (ALT) and HBV viral load is not always enough to correctly categorize the disease stage because of frequent fluctuations of both markers. Noninvasive tools such as serum markers and liver stiffness have been tested as complementary information to determine HBV infection

**Keywords:** Hepatitis B virus; Inactive carrier; Liver stiffness; HBV DNA; Quantitative HBsAg; Core-related antigen.

**Abbreviations:** ALT, alanine aminotransferase; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristic; CHB, chronic hepatitis B; C-index, concordance index; FIB-4, fibrosis-4 index; GGT, gamma glutamyl transferase; GZ, gray zone; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HR, hazard ratio; IC, inactive carriers; IQR, interquartile range; qHBsAg, quantitative hepatitis B surface antigen; LLD, lower limit of detection; LLQ, lower limit of quantification; LSM, liver stiffness measurement; ULN, upper limit of normal.

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phase.[5,6] However, the accuracy of these markers is not well standardized and some of them, such as quantification of hepatitis B surface antigen (HBsAg), have been shown to be deeply influenced by the HBV genotype.[7,8] These factors make difficult an accurate assessment and prediction of the long-term outcome of HBeAg-negative subjects by a single evaluation. As a result, a follow-up of three medical visits within the first year is currently recommended to define the phase of infection.[9] Although that approach reflects the changing character of the infection, one-point assessments should be developed in real-life cohorts to facilitate decentralized models of care and simplified algorithms. One-point assessments are especially important because of the endemicity of HBV infection in vulnerable populations and in low and middle-resource regions such as Sub-Saharan Africa, the Western Pacific, and Southeast Asia, which account for more than 80% of infections worldwide.[1,10] A proper classification is essential to identify not only individuals at increased risk of disease progression, but also those in an inactive phase of infection who would benefit from less intensive management strategies. The aim of this study was to assess the usefulness of noninvasive markers to identify subjects with HBeAg-negative chronic infection, namely former inactive carriers (ICs), and to develop a predictive model for early identification of these subjects in a single-point evaluation.

## Methods

A retrospective-prospective cohort study was performed in a university hospital in Barcelona, Spain. Subjects aged over 16 years of age with HBsAg documented for at least 6 months who attended the outpatient department between July 2013 and December 2019 were included. Subjects who were lost follow-up after the first medical visit were excluded. Subjects who tested positive for HBeAg at the first visit and/or with hepatitis C virus (HCV), hepatitis D virus, and human immunodeficiency virus coinfection defined as positivity for both serology and viral load, history of alcohol abuse and/or evidence of autoimmune liver disease were also excluded. Demographic, clinical, and anthropometric variables were collected in the first medical visit. Laboratory data and noninvasive markers of liver fibrosis were recorded at baseline, 6 months, and yearly. Hepatitis B infection parameters included quantitative HBsAg (qHBsAg). The lower limit of quantification (LLQ) was 0.05 IU/mL, HBeAg, and anti-HBe, all tested by commercially available electrochemiluminescence immunoassays (COBAS 8000; Roche Diagnostics, Rotkreuz, Switzerland). Serum HBV DNA was measured with a commercial PCR assay that had an LLQ of 20 IU/mL and lower limit of detection (LLD) of 10 IU/mL (COBAS 6800; Roche Diagnostics, Manheim, Germany). HBV core-related antigen (HBcrAg) was measured with a chemiluminescent enzyme immunoassay (CLIA, Lumipulse G HBcrAg assay; Fujirebio, Gent, Belgium) that had an LLD of 3 logU/mL. For HBV genotyping, HBV DNA was first enriched by ultracentrifugation of 9.6 mL of serum and Sanger sequencing was carried out after amplification of two different viral regions, PreC/Core (nucleotides 1,774–2,389, 615 bp) and PreS/Surface (nucleotides 2,828–176,561 bp), as previously described.[11] Genotypes H and F were combined because of their phylogenetic proximity and similar geographic distribution.[11,12] Noninvasive fibrosis markers included liver stiffness measurement-LSM (FibroScan) and the serum biomarkers Fibrosis-4 index (FIB-4) and the aspartate aminotransferase (AST)-to-platelet ratio index (APRI).

According to the one-time assessment at the first study visit, HBeAg-negative subjects were preclassified in three groups:

- Normal ALT and HBV DNA <2,000 IU/mL;
- ALT > two-fold upper limit of normal (ULN) and HBV DNA > 20,000 IU/mL;
- Subjects who did not fulfill any of the above conditions.

The ALT ULN was defined by local reference laboratory values of 35 IU/mL for women and 50 IU/mL for men. Subjects were followed by different hepatologists according to the same protocol. Following the guideline recommendations, liver biopsies were performed in subjects with HBV DNA persistently above 2,000 IU/mL and normal ALT or ALT <2-fold the ULN during follow-up.[13] Liver specimens were read by the same pathologist. Significant fibrosis was established in fibrosis stage ≥3 according to the Ishak score.[14] Subjects with at least one follow-up visit were reclassified, taking serum ALT, HBV DNA levels into consideration, and liver fibrosis stage by histological sample when needed, following European Association for the Study of the Liver 2017 Clinical Practice Guidelines.[13,14]

Chronic HBeAg-negative infection ICs was persistently normal ALT and HBV DNA of <2,000 IU/mL or HBV DNA between 2,000–20,000 IU/mL in the absence of significant fibrosis in liver biopsy.

Chronic HBeAg-negative hepatitis (CHB) was elevated ALT and HBV DNA >2,000 IU/mL, and/or significant fibrosis.

Gray zone (GZ) was persistently normal ALT and HBV DNA >20,000 IU/mL in the absence of significant fibrosis.

Liver cirrhosis was diagnosed by either imaging findings (irregular liver surface and direct/indirect signs of portal hypertension) or liver histology with an Ishak fibrosis score of 5–6. Subjects with liver ultrasound and/or histological signs of liver cirrhosis were considered as having CHB infection, regardless of their ALT and HBV DNA levels. Supplementary Figure 1 summarizes the study design. Participant data were anonymized and informed consent was waived because of the study design. The preparation of this manuscript was performed following STROBE guidelines.

### *Statistical analysis*

Quantitative variables with a normal distribution were reported as means and standard deviation. Non-normally distributed quantitative variables were reported as medians and interquartile range (IQR). Comparisons were performed with Student *t*-test and Mann-Whitney U-test. Categorical variables were described as absolute and relative frequencies (percentages, %) and compared with chi-square or Fisher's exact tests in case of relative frequencies below 5%. Baseline variables that had a clinically and statistically significant association to the outcome in univariate analysis (Mantel–Cox test) were selected for the initial models ($p<0.10$). The final models were obtained by a stepwise forward method based on model likelihood ratios (Cox regression). The same significance level ($p<0.05$) was set for including and discarding variables. Quantitative variables included in the models were categorized by clinically significant cutoffs in order to increase the power. The model obtained was calibrated by a 1000-bootstrapping analysis to minimize overfit bias.[15] A weighted semiquantitative score was constructed based on the final model. The score for each variable reflected the risk coefficient obtained after the bootstrapping analysis. The discrimination performance of the obtained predictive models was evaluated with receiver operating characteristic (ROC) curve analysis and the concordance index (C-index). The cutoff values were selected considering the highest Youden's index, and expressed as sensitivity, specificity, and predictive value. The results were considered statistically significant when the *p*-value was <0.05. The statistical analysis

**Table 1. Baseline characteristics in the overall cohort and the final classification**

| | Overall (*n*=343) | Inactive carriers (*n*=298) | Gray zone (*n*=9) | Chronic hepatitis B (*n*=36) | *p*-value |
|---|---|---|---|---|---|
| Male gender | 203 (59.2%) | 173 (58.1%) | 4 (44.4%) | 26 (72.2%) | 0.174 |
| Age (years) | 44.5±14.6 | 44.9±14.4 | 38.2±17.5 | 43.4±15.1 | 0.360 |
| Ethnicity | | | | | |
| Caucasian | 217 (63.3%) | 194 (65.1%) | 4 (44.4%) | 19 (52.8%) | 0.060 |
| Black | 79 (23.0%) | 63 (21.1%) | 5 (55.6%) | 11 (30.6%) | 0.060 |
| Asian | 23 (6.7%) | 18 (6.0%) | 0 (0%) | 5 (13.9%) | 0.060 |
| Hispanic | 24 (7.0%) | 23 (7.7%) | 0 (0%) | 1 (2.8%) | 0.060 |
| Comorbidities | | | | | |
| Obesity | 60 (17.5%) | 56 (23.0%) | 1 (11.1%) | 3 (10.7%) | 0.240 |
| Dyslipidemia | 53 (15.5%) | 51 (17.5% | 1 (11.1%) | 1 (2.8%) | 0.075 |
| Arterial hypertension | 53 (15.5%) | 48 (16.1%) | 1 (11.1%) | 4 (11.1%) | 0.685 |
| Diabetes mellitus | 14 (4.1%) | 11 (3.7%) | – | 3 (8.3%) | 0.339 |
| Liver cirrhosis | 8 (2.3%) | – | – | 8 (22.2%) | <0.001 |
| Platelets ($\times 10^9$/mm$^3$) | 225±58,000 | 228±57 | 245±58 | 198±58 | 0.009 |
| ALT (IU/L) | 28±21 | 25±11 | 29±15 | 56±46 | <0.001 |
| HBV DNA (log IU/mL) | 2.8±1.2 | 2.6±1.0 | 3.4±0.9 | 4.4±1.3 | <0.001 |
| qHBsAg (log IU/mL) | 3.1±1.1 | 3.0±1.1 | 3.9±0.6 | 3.7±0.6 | <0.001 |
| qHBsAg >1,000 IU/mL[1] | 205 (60.5%) | 168 (56.9%) | 8 (88.9%) | 29 (82.9%) | 0.003 |
| HBcrAg (log U/mL)[2] | | | | | |
| <3 logU/mL | 274 (79.9%) | 258 (91.5%) | 4 (44.4%) | 12 (37.5%) | <0.001 |
| 3–4 logU/mL | 38 (11.1%) | 23 (8.2%) | 4 (44.4%) | 11(34.4%) | <0.001 |
| 4–5 logU/mL | 8 (2.3%) | 1(0.4%) | 1 (11.1%) | 6 (18.8%) | <0.001 |
| >5 logU/mL | 3 (0.9%) | – | – | 3 (9.4%) | <0.001 |
| Genotype[3] | | | | | |
| D | 102 (40.8%) | 93 (43.1%) | 2 (22.2%) | 7 (28.0%) | 0.209 |
| A | 68 (27.2%) | 61 (28.2%) | 2 (22.2%) | 5 (20.0%) | 0.209 |
| E | 40 (16.0%) | 31 (14.4%) | 3 (33.3%) | 6 (24.0%) | 0.209 |
| F/H | 26 (10.4%) | 20 (9.3%) | 2 (22.2%) | 4 (16.0%) | 0.209 |
| B/C | 10 (4.0%) | 7 (3.2%) | – | 3 (12.0%) | 0.209 |
| Mixed | 4 (1.6%) | 4 (1.9%) | – | – | 0.209 |
| Elastography (kPa) | 5.6±2.3 | 5.2±1.7 | 6.9±1.5 | 8.2±4.3 | <0.001 |
| FIB-4 | 0.5±0.4 | 0.5±0.4 | 0.4±0.2 | 0.5±0.7 | 0.617 |
| APRI | 0.5±0.4 | 0.4±0.2 | 0.5±0.3 | 0.9±0.9 | <0.001 |

Categorical variables are *n* (%), quantitative variables are means ± SD. [1]qHBsAg was available in 339 subjects of the overall cohort (295 inactive carriers, nine gray zone, 35 chronic hepatitis); [2]HBcrAg was available in 323 subjects; [3]HBV-genotype was available in 250 subjects of the overall cohort (216 inactive carriers, nine gray zone, 25 chronic hepatitis B). ALT, alanine aminotransferase; APRI, ALT to platelet ratio index; FIB-4, fibrosis-4 index; HBV, hepatitis B virus; HBcrAg, hepatitis B core-related antigen; qHBsAg, quantitative hepatitis B surface antigen.

were performed with IBM SPSS, version 26.0 (IBM Corp, Armonk, NY, USA).

## Results

### *Baseline characteristics*

Three hundred forty-three consecutive subjects were included (Table 1). Most were male (59.2%), Caucasian (63.3%), and the mean age was 45 years. Black individuals represented a considerable percentage of the overall cohort (23.0%), most of them coming from Western African countries, and 179 subjects (52.1%) were immigrants from non-Western European regions. The HBV genotype was determined in 250 individuals; D and A were the most prevalent, followed by E and H/F. Figure 1 summarizes the country of origin and the most prevalent HBV genotypes among immigrants. At the first visit, most subjects (68.8%) had a

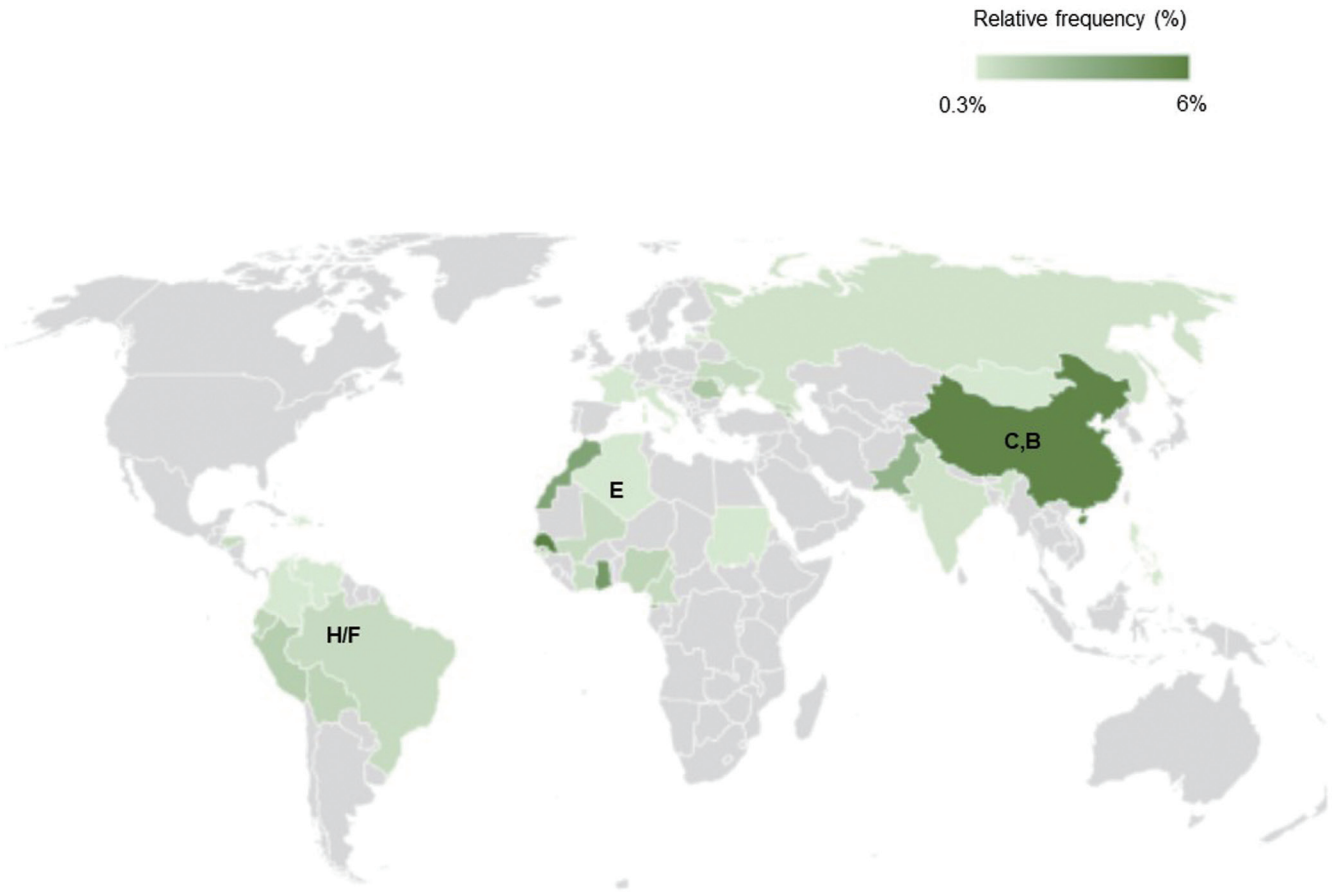


**Fig. 1. Country of origin and most prevalent HBV genotype of immigrants in the overall cohort (by relative frequency).** HBV, hepatitis B virus.

normal ALT and HBV DNA <2,000 IU/mL, five (1.5%) had an ALT >2× the ULN and HBV DNA >20,000 IU/mL, and 102 (29.7%) did not fulfill the conditions for any of the above categories (Fig. 1).

### *Clinical follow-up and liver biopsy*

Two hundred fifty-four (74.1%) subjects were classified by a noninvasive approach, and liver biopsies were needed in 89 (25.9%) for proper classification of the HBV phase. Twenty-eight (12.1%) of two hundred thirty-one subjects presented with baseline normal ALT and an HBV DNA <2,000 IU/mL, and sixty-one (61.0%) of 100 were initially considered as GZ. The median time from the first visit to liver biopsy was 8.4 months (IQR 2.2–15.3). Nine out of 89 (10.1%) subjects presented significant fibrosis (≥F3), whereas 73 (82.0%) presented Ishak score below F1. Table 2 summarizes baseline features by fibrosis stage, in subjects who underwent invasive management. Significant fibrosis was significantly more frequent in males, whereas no differences were found in baseline ALT, qHBsAg and HBV-DNA levels. However, significantly higher baseline HBcrAg levels were observed in subjects with significant fibrosis ($p$<0.001). LSM tended to be lower among subjects without significant fibrosis ($p$=0.085). However, when categorizing liver stiffness measurements with a double cutoff system (i.e. below 6.5 kPa, between 6.5 and 9 kPa and above 9 kPa), a significant association was found between elastography categories and the presence of significant fibrosis in liver samples ($p$=0.05, Supplementary Fig. 2).[5,16] FIB-4 values did not differ in the presence of significant fibrosis, but those with ≥F3 had higher APRI levels ($p$=0.048). Multivariate analysis found that LSM categories (HR 3.37, 95% CI: 1.08–10.49, $p$=0.037) and baseline HBcrAg (HR 3.615, 95% CI: 1.41–9.25, $p$=0.007) were independently associated with significant fibrosis.

### *Classification of the HBV phase during follow-up*

Subjects were classified after a mean follow-up of 39.0 months. Figure 2 shows the changes from the initial to the final classification. At follow-up, 298 subjects were considered ICs, 36 patients were found to have CHB, and nine subjects remained in in the GZ, all had viral loads persistently over 20,000 UI/mL, normal ALT, and non-significant fibrosis on liver biopsy. Of the 236 subjects with normal ALT and HBV DNA <2,000 IU/mL, 230 (97.5%) were considered ICs during follow-up and six (2.5%) were regarded as CHB after the histological assessment. Of 102 subjects in the GZ group after the one-point assessment at baseline, 68 (66.7%) were finally classified as ICs and 25 (24.5%) as CHB. All five subjects with initial ALT levels >2 times the ULN and HBV DNA >20,000 IU/mL remained a the CHB stage.

Baseline features by final classification are summarized in Table 1. Ethnic distribution tended to differ ($p$=0.060), with a higher proportion of black individuals in the GZ (55.6%). The prevalence of transmission pathways, toxic habits,

**Table 2. Baseline features of individuals who required a liver biopsy during follow-up for proper classification of HBV infection**

| | Non-significant fibrosis (<F3), (*n*=80) | Significant fibrosis (≥F3), (*n*=9) | Univariate analysis |
|---|---|---|---|
| | | | *p*-value |
| Male gender | 39 (48.8%) | 8 (88.9%) | 0.023 |
| Age (years) | 44±13 | 41±15 | 0.510 |
| Ethnicity | | | |
| Caucasian | 48 (60%) | 5 (55.6%) | 0.832 |
| Black | 21 (26.3%) | 3 (33.3%) | 0.832 |
| Asian | 6 (7.5%) | 1 (11.1%) | 0.832 |
| Hispanic | 5 (6.3%) | 0 (0%) | 0.832 |
| Platelets ($\times 10^9$/mL) | 223±56 | 203±35 | 0.295 |
| ALT (IU/L) | 30±16 | 39±9 | 0.130 |
| qHBsAg (log IU/mL) | 3.6±0.7 | 3.5±0.6 | 0.673 |
| qHBsAg >1,000 IU/mL | 63 (79.7%) | 8 (88.9%) | 0.447 |
| HBcrAg (log U/mL) | | | |
| <3 logU/mL | 64 (81.0%) | 5 (62.5%) | |
| 3–4 logU/mL | 11 (13.9%) | – | |
| 4–5 logU/mL | 4 (5.1%) | – | |
| >5 logU/mL | – | 3 (37.5%) | |
| HBV DNA (log IU/mL) | 3.6±0.9 | 4.2±1.3 | 0.079 |
| Genotype | | | |
| D | 23 (37.1%) | 2 (40%) | 0.139 |
| A | 16 (25.8%) | 0 (0%) | 0.139 |
| B/C | 3 (4.8%) | 0 (0%) | 0.139 |
| F/H | 10 (16.1%) | 0 (0%) | 0.139 |
| E | 10 (16.1%) | 3 (60%) | 0.139 |
| Elastography (kPa) | 6.1±2.3 | 7.5±1.9 | 0.085 |
| FIB-4 | 0.4±0.2 | 0.3±0.1 | 0.092 |
| APRI | 0.4±0.2 | 0.6±0.2 | 0.048 |

Categorical variables are *n* (%), quantitative variables are means±standard deviation. ALT, alanine aminotransferase; APRI, ALT to platelet ratio index; FIB-4, fibrosis-4 index; HBcrAg, hepatitis B core-related antigen; qHBsAg, quantitative hepatitis B surface antigen; HBV, hepatitis B virus.

and comorbidities (i.e. diabetes mellitus, arterial hypertension, and dyslipidemia) was similar in all three phases of infection. Significant differences were found in baseline ALT ($p$<0.001) HBV DNA ($p$<0.001), qHBsAg ($p$<0.001) and HBcrAg levels ($p$<0.001), as well as LSM and APRI at first visit; no differences were found in FIB-4 score ($p$=0.617). Genotype distribution was not significantly different in the three phases of infection ($p$=0.209).

### *Markers for identification of HBV ICs*

Baseline markers associated with IC in the univariate analysis were lower levels of ALT, AST, gamma glutamyl transferase (GGT), higher platelet count, lower HBcrAg, HBV DNA, and LSM. Independent association was confirmed in the multivariate analysis between the IC group and lower HBV DNA, HBcrAg levels and LSM (Table 3). Risk coefficients similar to those obtained by multivariate analysis were obtained by 1000 bootstrapping samples, HR 6.0 (95% CI: 3.0–12.0), $p$<0.001 for categorized HBV DNA, HR 4.6 (95% CI: 2.3–9.0), $p$<0.001, for LSM; and HR 6.5 (95% CI: 2.7–15.7, $p$<0.001) for HBcrAg. An ROC model based on these coefficients yielded an AUROC of 0.925 (95% CI: 0.880–0.970, $p$<0.001) for the identification of ICs (Fig. 3). The model was validated in most prevalent HBV genotypes and had an area under ROC (AUROC) of 0.95 for genotype D, 0.96 for A, 0.90 for E and 0.88 for H/F (Table 4). An individual-score system, the ACE score, was constructed from simplified coefficients in the bootstrapping analysis (Table 5) and included HBV DNA, HBCore-related antigen, and liver elastography). The ACE score had the highest positive predictive value for identification of ICs for patients with punctuations <1 point, and 12 points was the cutoff with the greatest diagnostic accuracy (93.8%). The accuracy of the different cutoffs of the ACE score are summarized in Table 6.

## Discussion

Herein we identified baseline LSM, HBV DNA, and HBcrAg

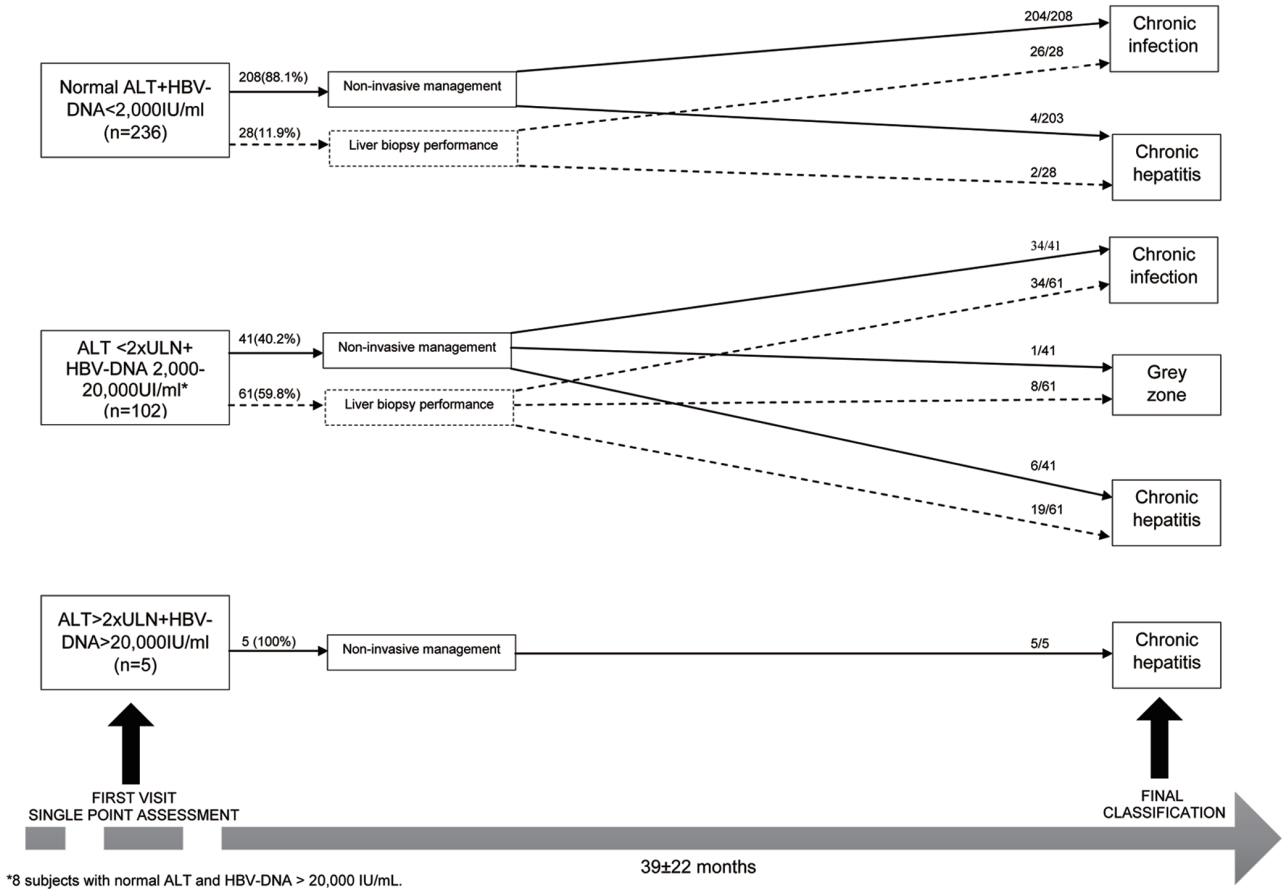


**Fig. 2. HBV infection phase evolution and liver biopsy performance during follow-up.** *Liver biopsy indication was established by normal ALT plus HBV DNA persistently above 2,000 IU/mL, or ALT <2-fold ULN plus viral load above 2,000 IU/mL during follow-up. **Final classification was carried out according to European Association for the Study of the Liver 2017 Clinical Practice Guidelines.[16] Chronic HBeAg-negative infection-inactive carriers had persistently normal ALT plus HBV DNA <2,000 IU/mL or HBV DNA between 2,000 and 20,000 IU/mL in the absence of significant fibrosis in liver biopsy. Chronic HBeAg negative hepatitis required elevated ALT and HBV DNA >2,000 IU/mL and/or significant fibrosis at liver biopsy. Gray zone required persistently normal ALT and HBV DNA >20,000 IU/mL in the absence of fibrosis in liver biopsy. ALT, alanine aminotransferase; HBV, hepatitis B virus; ULN, upper limit of normal.

**Table 3. Univariate and multivariate COX proportional regression analysis of baseline factors associated with the follow-up classification as chronic infection-inactive carriers**

| ***n*=336** | **Univariate analysis** | **Multivariate analysis** | |
|---|---|---|---|
| | ***p*-value** | **HR (95%CI)** | ***p*-value** |
| Male sex | 0.176 | – | – |
| Caucasian ethnicity | 0.051 | – | 0.378 |
| Age | 0.288 | – | – |
| ALT (IU/L) | <0.001 | – | 0.179 |
| AST (IU/L) | <0.001 | – | 0.323 |
| GGT (IU/L) | <0.001 | – | 0.269 |
| Platelets (cells/mL) | 0.029 | – | 0.996 |
| qHBsAg (log IU/L)[1] | <0.001 | – | 0.111 |
| HBV DNA (by category)[2] | <0.001 | 5.9 (2.9–11.9) | <0.001 |
| HBcrAg (log U/mL)[3] | <0.001 | 6.3 (2.6–15.1) | <0.001 |
| Elastography (by category)[2] | <0.001 | 4.0 (2.0–7.9) | <0.001 |

[1]qHBsAg was available in 339 subjects of the overall cohort; [2]HBcrAg was available in 323 subjects; [3]Categories were introduced according to their most commonly used cutoffs: HBV DNA <2.000 IU/mL, 2,000–20,000 IU/mL, >20.000 IU/mL; liver stiffness: <6.5 kPa, 6.5–9 kPa, >9 kPa. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase; HBcrAg, hepatitis B core-related antigen; qHBsAg, quantitative hepatitis B surface antigen; HBV, hepatitis B virus.

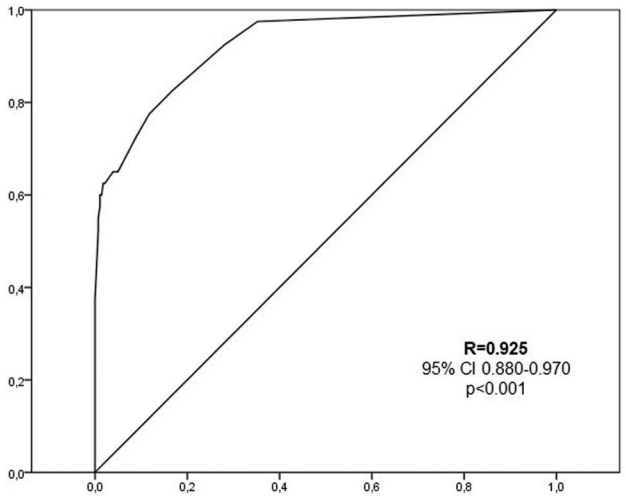


**Fig. 3. Area under the receiver operating characteristic (AUROC) of the model for identification of HBeAg-negative chronic infection-inactive carriers subjects.**

values as independent predictors for the identification of HBV ICs. We performed a retrospective-prospective real-life cohort study, with the development of a scoring system (ACE score) that combined the baseline variables. The score had a high specificity and positive predictive value, which implies a trustworthy identification of IC subjects with a low risk of disease progression and good performance regardless of the HBV genotype.

Classically, efforts have been made to identify HBV-infected subjects at increased risk of developing liver-related complications,[17] as antiviral treatment with high-barrier nucleos(t)ides analogues is effective, affordable, and has a great impact on disease progression and survival.[18,19] However, a more recent approach focuses not only on identifying subjects at increased risk, but also those in the inactive phase of the disease who would benefit from less intensive follow-up and management.[20,21] Individuals in this phase have benign outcomes with morbidity and mortality similar to those of the general population.[22] An easy and accurate identification of ICs in a single assessment would facilitate decentralization strategies for HBV follow-up.

Many recent studies have approached the identification of ICs using HBV biomarkers and their combination. HBsAg titles have been proposed as a useful tool to identify IC because of their correlation with HBV DNA levels. An algorithm based on a single-point determination of qHBsAg, ALT, and HBV DNA was described in a large Taiwanese cohort of HBeAg-negative subjects with HBV DNA 2,000 IU/mL (ERADICATE-B cohort). The algorithm proposed the use of qHBsAg <1,000 IU/mL for the identification of subjects at minimal risk of disease progression, but its application was limited to HBV-genotype B and C.[23] The same cutoff was proposed in an Italian cohort of subjects infected with genotype D.[24] However, it is difficult to generalize the results for the overall HBV population, as qHBsAg has been shown to significantly vary among different HBV genotypes, which limits its application in genotype-diverse cohorts.[7,11] HBcrAg was later postulated as a surrogate marker of intrahepatic cccDNA.[25] Significant variation in HBcrAg levels was detected throughout the different HBV infection phases,[11] with the lowest titers detected in the ICs.[26] Interestingly, in another study including HBV genotypes E and H/F, HBcrAg <3 logU/mL combined with HBV DNA <2,000 IU/mL had a diagnostic accuracy of 85% for identification of ICs regardless of HBV genotype.[11] Recently, a multicenter European study including 1,582 HBeAg-negative subjects, HBcrAg <3 logU/mL had an AUROC of 0.968 for identification of ICs.[27] LSM in HBV infection is not as well standardized as in HCV. Double cutoff systems have been proposed to improve performance, although there is no consensus among the different guidelines, which hinders its application in daily practice.[28,29] An Italian study reported a combination of HBsAg, LSM, and HBV DNA with 100% specificity, but no data regarding HBV genotype were available.[30] In fact, to the best of our knowledge, no algorithms including LSM have been developed and validated in all HBV genotypes.

On the other hand, the inaccuracy of noninvasive fibrosis markers in the GZ usually leads to the necessity of performing a liver biopsy, which is considered the gold standard for fibrosis assessment. In our study roughly 25% of patients needed a liver biopsy, 10% of whom had significant fibrosis that was independently associated with higher HBcrAg levels and LSM. The relatively high percentage of patients who required a liver biopsy for classification of HBV phase, reinforces the need to optimize the use of noninvasive strategies and to develop pan-genotypic scores.

Our cohort included mainly middle-aged subjects in the IC phase, which is consistent with the current epidemiological profile of the HBV infection in Western countries.[31] On the other hand, almost half of our cohort were non-European migrants, which probably explains the genotype distribution compared with other European cohorts.[26,32] The high proportion of migrants in our cohort should be a reminder of the need of the integration of viral hepatitis management

**Table 4. Area under the receiver operating characteristic (AUROC) of the model for inactive carrier identification by HBV genotype**

| Genotype | *n* | AUROC | 95% CI | *p*-value |
|---|---|---|---|---|
| | | | | |
| D | 98 | 0.955 | 0.91–1.0 | <0.001 |
| A | 67 | 0.963 | 0.89–1.0 | <0.001 |
| E | 37 | 0.903 | 0.79–1.0 | <0.001 |
| H/F | 26 | 0.883 | 0.75–1.0 | 0.005 |

AUROC, area under the receiver operating characteristic; CI, confidence interval.

**Table 5. Score system based on the novel model**

| | Score |
|---|---|
| HBV DNA (IU/L) | |
| <2,000 IU/L | 0 |
| 2,000–20,000 IU/L | 6 |
| >20,000 IU/L | 12 |
| Liver stiffness (kPa) | |
| <6.5 kPa | 0 |
| 6.5–9.0 kPa | 5 |
| >9.0 kPa | 10 |
| HBcrAg (logU/mL) | |
| ≤3 logU/mL | 0 |
| 3–4 logU/mL | 6 |
| 4–5 logU/mL | 12 |
| >5 logU/mL | 18 |

HBcrAg, hepatitis B core-related antigen.

**Table 6. Accuracy of the ACE score cutoffs for identification of subjects who will be considered HBeAg-negative chronic infection (inactive carriers) during follow-up**

| Total score | <1 point | ≤5 points | ≤12 points | ≤17 points |
|---|---|---|---|---|
| Sensitivity, % (95% CI) | 64.8 (59.0–70.1) | 71.9 (66.4–76.8) | 98.2 (95.9–99.2) | 99.3 (97.5–99.8) |
| Specificity, % (95% CI) | 97.5 (87.1–99.6) | 92.5 (80.4–97.4) | 62.5 (47.0–75.8) | 52.5 (37.5–67.1) |
| Negative predictive value, % (95% CI) | 28.3 (21.4–36.3) | 31.9 (24.1–40.9) | 83.3 (66.4–92.7) | 91.3 (73.2–97.6) |
| Positive predictive value, % (95% CI) | 99.5 (97.0–99.9) | 98.5 (95.8–99.5) | 94.9 (91.7–96.9) | 93.6 (90.3–95.9) |
| Diagnostic accuracy, % (95% CI) | 68.9 (63.6–73.7) | 74.5 (66.4–78.9) | 93.8 (90.6–95.9) | 93.5 (90.2–95.7) |

CI, confidence interval.

in the package of care of international health units, as well as of the need of predictive models that include a diverse genotype composition.

Our study has some limitations because of the partially retrospective design, which could lead to bias resulting from missing parameters such as HBcrAg and qHBsAg in some patients. Furthermore, as previously mentioned, the proportion of patients with CHB was relatively low, but was in line with the prevalence shown by other studies carried out in Europe.[11,33] The external validation of our findings is limited by the unicentric character of the study, although patients were followed up by different hepatologists. Also, a limited number of Asian and Hispanic subjects were included in the cohort. Regardless of the limitations, the new model performed well and could be a valuable tool in the clinical practice following validation in large multicenter cohorts.

In summary, in patients with chronic HBV infection, low levels of HBV DNA, HBcrAg, and LSM were independently associated with the inactive carrier state. The ACE score included these variables and accurately identified ICs in a single-point time evaluation, regardless of HBV genotype. Studies in larger cohorts are needed to validate the score.

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## Conflict of interest

Mar Riveiro-Barciela (MRB) and Rafael Estebal (RE) have served as speakers for AbbVie and Gilead. MRB and María Buti (MB) have received grants from Gilead. MB has served as speaker for Abbvie and Gilead and advisory board member for Gilead, Assembly, GSK.

## Author contributions

Guarantors of the article and take responsibility for the integrity of the work (MB, MRB), designed the study (MB, MRB, LR), collected the data (LR, AP, EVA, AR, MB, RC, SS, DT), performed the analysis and interpretation (LR, MRB, MB), drafted the manuscript (LR, MRB, MB), reviewed the manuscript (RE, FRF). All the authors approved the final version.

## Ethical statement

Ethical approval was obtained from the Ethical Committee of the University Hospital Vall d'Hebron (Code PR(AG) 247/2018; Date of approval 20 July 2018).

## Data sharing statement

The data used to support the findings of this study are available from the corresponding author upon request.

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